Survival and Development of *Heliothis virescens* (Lepidoptera: Noctuidae) Larvae on Isogenic Tobacco Lines with Different Levels of Alkaloids

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ABSTRACT Levels of pyridine alkaloids were measured in 18 tobacco, *Nicotiana tabacum* L., entries from three parental isolines ('NC 95', 'SC 58', and 'Coker 139'), grown at Tifton, GA, Florence, SC, and Oxford, NC, in 1991. Levels of alkaloids in bud leaves (first fully unfolded leaf below the apical leaf bud) were negatively correlated to natural infestation ratings of tobacco budworm larvae, *Heliothis virescens* (F.), 7 wk after transplanting. For artificially infested bud leaves at Oxford, there was a significant negative correlation between levels of total alkaloids and larval weights after 1 wk of feeding. In 1992, four entries from the 'NC 95' isoline were grown at Oxford, and samples for alkaloid analyses were taken every 2 wk at several leaf positions on each plant. During weeks 4, 8, 12, and 16, second instar tobacco budworms were caged on individual, intact leaves inside perforated plastic bags in the field. The survival and development of tobacco budworm larvae after 1 wk were negatively correlated with levels of alkaloids at the various leaf positions. Larvae survived better and grew faster on the bud leaves of each entry where alkaloid levels were lower than they did on leaves further down the stalk where alkaloid levels were higher. More larvae survived on the lower leaves of the low alkaloid lines than on the lower leaves of the high alkaloid lines. Even moderate increases in pyridine alkaloids had negative effects on tobacco budworm survival and development. Nicotine constituted >97% of the pyridine alkaloids in the 'NC95' isoline each year.

KEY WORDS tobacco budworm, *Nicotiana*, nicotine, anabasine, nornicotine, anatabine

THE PYRIDINE ALKALOIDS are a characteristic of plants in the genus *Nicotiana* of Solanaceae (Shmuck et al. 1941, Jeffrey 1959, Saitoh et al. 1985, Bush and Crowe 1989, Sisson and Severson 1990). Nicotine is the most prevalent of these compounds, and it typically makes up >95% of the total alkaloids in commercial flue-cured tobacco, *Nicotiana tabacum* L. (Sisson and Severson 1990). The insecticidal properties of nicotine have been known since at least 1690 (Schmeltz 1971). Throughout the 18th century, crude aqueous extracts or dusts from tobacco were recommended for control of insect pests (Metcalf et al. 1962). The primary active ingredient, nicotine, was isolated and named in 1828 (Schmeltz 1971). Other pyridine alkaloids found in *Nicotiana* spp., such as anabasine, anatabine, and nornicotine, also have insecticidal properties (Metcalf et al. 1962).

Self et al. (1964) reported that tobacco budworm larvae, *Heliothis virescens* (F.), do not metabolize tobacco alkaloids, but they are able to excrete these compounds to some extent. At topical doses of 200 μg of free base nicotine in 1 μl of acetone, larvae showed no symptoms of poisoning. However, Granzow et al. (1985) demonstrated that first instar tobacco budworms were adversely affected by higher concentrations of nicotine in an artificial diet. Both survival and rate of growth were lowered, and an LC_{50} of 0.67% (dry weight) was determined. There was some retardation of growth with nicotine concentrations as low as 0.25% dry weight, which is well within the range for ripe leaves of commercial flue-cured tobacco cultivars (2–4% nicotine) (Sisson and Severson 1990, Tso 1990). Gunasena et al. (1990) reported that low doses of nicotine applied to artificial diet reduced the weight of tobacco budworm neonates and prolonged their developmental time. For 7-d feeding experiments, larval weights were reduced by 50% (ED_{50}) by an estimated nicotine concentration of 0.007% wet weight. High nicotine concentrations in artificial diets also reduce pupal weights of tobacco budworms (Krafft and Klingauf 1981, Gunasena et al. 1990).

Girardeau et al. (1973) related damage to nicotine content, trichome density, and trichome exudates by tobacco budworm larvae in flue-cured tobacco. Severson et al. (1981) reported on the alkaloid levels of six tobacco entries that were resistant to tobacco budworm larvae. The alkaloid levels (93–97% nicotine) for green leaf buds were up to five times higher in the

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resistant tobaccos than in 'NC 2326', a typical flue-cured tobacco. Lower larval damage in the resistant tobacco entries may have been due, in part, to the high alkaloid levels. Also, the induction of nicotine in response to herbivory has been described for a number of *Nicotiana* species (Baldwin 1988, 1989, 1994, 1999; Baldwin and Ohnmeiss 1993).

We were interested the effects of pyridine alkaloids from flue-cured tobacco on the survival and development of tobacco budworm larvae in the field. To study this, we investigated the survival and growth of larvae on isogenic tobacco lines that varied mainly in levels of pyridine alkaloids, primarily nicotine.

Materials and Methods

The tobacco budworm larvae used in this study were from laboratory colonies that had been started from egg collections from flue-cured tobacco near Oxford, NC, in the fall of 1990 and 1991. The laboratory colonies were maintained on an artificial diet (Baumhover 1985) for 7–10 generations before insects were used. Neonate larvae were fed for 2 d on the same artificial diet before being used as second instars in field experiments.

1991 Experiments. Eighteen tobacco genotypes from three parental lines were grown in 12-plant plots in a randomized complete block design with three replications each at Tifton, GA, Florence, SC, and Oxford, NC. Chaplin and Burk (1984) developed these tobacco entries as isolines varying primarily in levels of alkaloids. They were derived from three parental lines: 'NC 95', 'SC 58', and 'Coker 139'. Total alkaloids in the cured leaves of these tobacco entries were reported to range from 0.20 to 4.82% (Chaplin and Burk 1984). The tobacco entries from the NC 95 isoline were LN-1 (=NC 95, a standard flue-cured tobacco; Chaplin and Burk 1984), LN-4 (=LMAFC 34; Chaplin 1984), LN-7 (=MAFC 5; Chaplin 1986), LN-10, and C-5 (=LAFC 53; Chaplin 1975). Tobacco entries from the SC 58 isoline were SC 58, L-22, LN-25, LN-28, LN-31, LN-34, and C-13. Tobacco entries from the Coker 139 isoline were LN-40, LN-43, LN-46, LN-49, C-20, and C-25 (=Coker 139). Plots were separated from other experiments by buffer rows of 'NC 2326'. Transplants were produced in tobacco plant beds by standard procedures for flue-cured tobacco (Chaplin et al. 1976, Hawks and Collins 1983).

Before transplanting, the soils at Tifton and Florence were treated with the recommended rates of metalaxyl (0.56 kg [AI]/ha; Ridomil 2E, Novartis Crop Protection, Greensboro, NC), pebulate (4.25 kg [AI]/ha; Tillam, Zeneca Agricultural Products, Wilmington, DE), napropamide (4.68 liter [AI]/ha; Devrinol 2E, Zeneca, Agricultural Products, Wilmington, DE), and fensulfothion-fenamiphos (9.36 liter [AI]/ha; Dasanit-Nemacur, Bayer Corp., Kansas City, MO) for control of diseases, weeds, nematodes, and soil insects, respectively. The soil at Oxford was treated with the recommended rates of metalaxyl (0.56 kg [AI]/ha) and isopropalin (1.59 kg [AI]/ha; Paarlan, Dow Agrosciences, Indianapolis) for control of fungal diseases and weeds, respectively. No foliar insecticides were used after transplanting at any location.

Tobacco was transplanted at Tifton on 26 April. The field was fertilized with 530 kg/ha of 6-6-18 (N-P-K) on 25 May and later topdressed with additional fertilizer (16-0-0). At Florence, the tobacco was planted on 10 April, fertilized (6-6-18) on 10 May, and later topdressed with additional fertilizer (16-0-0). Tobacco was transplanted on 17 May and fertilized (8-8-24) on 24 May at Oxford. The Oxford field was cultivated and topdressed with additional fertilizer (15-0-14) on 6 June.

Ten wk after transplanting at Oxford and Florence, one-half of the plants in each plot were topped (removal of apical meristem and flowers, a normal production practice) (Chaplin et al. 1976, Hawks and Collins 1983) so that ≈20 (range, 18–22) leaves remained. Topping stimulates the plant to reallocate energy into leaf maturation (Hawks and Collins 1983, Tso 1990), but it also promotes the production of axillary leaf buds or suckers (Chaplin et al. 1976, Tso 1990). Suckers were removed by hand from each plant each week. The other one-half of the plants in each plot were cut off just above the ground (ratooned), and these cutoff plants were allowed to regrow from a single axillary leaf bud. Ratooned plants produce new leaves that are similar in physical and chemical characteristics to leaves from the original growth (Jackson et al. 1987).

The Tifton and Florence plots were sampled for alkaloids 3, 5, and 7 wk after transplanting. In addition, the Florence plots were sampled 5 wk after topping (13 wk after transplanting). The plots at Oxford were sampled for alkaloids every 2 wk from plant bed (week 0) through the final harvest of the leaves for curing (week 18). Five bud leaves (first fully unfolded leaf below the apical leaf bud) were removed from each plot, and two 2-cm diam leaf plugs (3.14 cm^2) were taken from the center, but not including the midvein, of each leaf. These leaf plugs were placed into scintillation vials containing 95% methanol and frozen immediately on dry ice. They were transported frozen to Athens, GA, where they were analyzed for alkaloids by the techniques of Severson et al. (1981).

At each location, a second set of 2-cm diam leaf plugs was taken from the same 10- to 20-cm long bud leaves that had been used for the alkaloid samples at 3–4 and 7–8 wk. Leaf plugs were weighed and slowly dipped 10 times into scintillation vials containing methylene chloride to remove cuticular components (Severson et al. 1984). The leaf plugs were then discarded. The scintillation vials containing the extracts were frozen on dry ice and shipped to Athens, GA, where the samples were analyzed for cuticular components by the techniques of Severson et al. (1984, 1985, 1988). The principal cuticular components of interest were the cembranoid diterpenes (=duvanes), (1*S*,4*S*,6*R*,2*E*,7*E*,11*E*)-4,6-dihydroxycembra-2,7,11-triene and its (4*R*) epimer, trivially known as α- and β-4,8,13-duvatriene-1,3-diols (α- and β-CBT-diols) (Roberts and Rowland 1962, Jackson and Danehower 1996). Also found at lower levels were (1*S*,4*S*,6*R*,2*E*, 7*E*,11*E*)-4-hydroxycembra-2,7,11-triene and its corresponding (4*R*) epimer, trivially known as α- and β-4,8,13-duvatrien-1-ols (α- and β-CBT-monols) (Wahlberg et al. 1981, Jackson and Danehower 1996).

The plots at Tifton and Florence were evaluated for tobacco budworm damage 6 and 7 wk after transplanting, respectively. Subjective damage ratings were made on a 0–7 scale, with 0 being no damage; 1, any noticeable damage; 3, light damage with some holes in the tobacco bud; 5, moderate damage with extensive feeding throughout the bud; and 7, severe damage with the bud severed from the plant. The ratings of 2, 4, and 6 were intermediate to the odd-numbered ratings. This rating system has been described previously (Jackson et al. 1985, Jackson and Severson 1989). For the data sets from Tifton and Florence, a regression analysis (PROC REG) (SAS Institute 1989) was performed for the relationship between levels of total alkaloids and average damage ratings.

The plots at Oxford had very low infestations of tobacco budworm larvae, so they were not scored for damage. Instead, at 6 and 8 wk after transplanting, five plants from each plot were artificially infested with five second instar tobacco budworms per plant using a fine brush. The top 4–5 leaves of inoculated plants were enclosed in perforated plastic bags (Del Net, Hercules, Wilmington, DE). The bags were opened and all surviving larvae were weighed after 1 wk. This technique has been used successfully in past studies (Jackson et al. 1987, Gwynn et al. 1990). A regression analysis (PROC REG) was performed for the relationship between levels of total alkaloids and average weight of tobacco budworm larvae after 1 wk of feeding. An analysis of the homogeneity of regression coefficients (Steel and Torrie 1960) was used to determine whether the slopes of the regression lines for each location were statistically different from each other.

1992 Experiments. In the second year of field studies, four tobacco entries were grown in 12-plant plots in a randomized complete block design with three replications at Oxford, NC. The four entries were from the NC 95 isoline. They were NC 95(=LN-1; 3.28% total alkaloids), LN-4 (1.08%), LN-7 (2.05%), and C-5 (0.34%) (Chaplin and Burk 1984). Before transplanting (24 April), the field was treated with isopropalin (1.59 kg [AI]/ha) and metalaxyl (0.56 kg [AI]/ha) to control weeds and fungal diseases, respectively. Transplants were produced in field plant beds by standard cultural techniques (Chaplin et al. 1976, Hawks and Collins 1983).

On 8 May, transplants were placed into a field of Helena sandy loam on 56-cm centers with ≈1.2 m between rows. Plots were fertilized (530 kg/ha of 8-8-24) on 27 May, topdressed (159 kg/ha of 15-0-14) on 2 June, and cultivated and irrigated according to standard cultural practices for flue-cured tobacco production (Chaplin et al. 1976, Hawks and Collins 1983). Ten wk after transplanting (17 July), one-half of the plants in each plot were topped, and all axillary leaf buds were removed by hand each week thereafter. The other one-half of the plants in each plot were cut off just above the ground, and these ratooned plants were allowed to regrow from a single axillary leaf bud (Jackson et al. 1987).

Five leaf positions were sampled for alkaloids and bioassayed for insect survival and development: (a) bud leaf or highest remaining leaf on topped plants, (b) leaf five or six from the top of the plant, (c) leaf nine or 10 from the top of the plant, (d) leaf 13 or 14 from the top of the plant, and (e) leaf 17 or 18 from the top of the plant. Five plants per plot were infested with second instar tobacco budworms at 4, 8, 12, and 16 wk after transplanting. Leaves for each leaf position were individually infested with five larvae, and each leaf was enclosed in a perforated plastic bag (Del Net). All surviving larvae were weighed after 1 wk. All plots were sampled for alkaloids 1 d before larval bioassays. Alkaloid samples were taken from plants adjacent to ones used for the insect bioassays. Five leaves were removed for each leaf position from each plot. Two 2-cm diam leaf plugs were taken from the center, but not including the midvein, of each leaf. These leaf plugs were weighed and then placed into scintillation vials containing methanol and frozen immediately on dry ice. They were transported frozen to Athens, GA, where they were analyzed for alkaloids by the techniques of Severson et al. (1981).

Data were analyzed by analysis of variance (ANOVA) using PROC GLM (SAS Institute 1989), and treatment means were separated by Fisher's least significant difference (LSD) test (SAS Institute 1989). Planned orthogonal polynomial comparisons (Steel and Torrie 1960, SAS Institute 1989) were used to partition sums of squares for individual comparisons of ratooned versus topped plants for weeks 12 and 16. Regression analyses (PROC REG) were performed for the relationships between levels of total alkaloids and average weight of tobacco budworm larvae after 1 wk of feeding on the tobacco isolines at weeks 4, 8, 12, and 16. An analysis of the homogeneity of regression coefficients (Steel and Torrie 1960) was used to determine whether the slopes of the regression lines for each week were statistically different from each other.

Results

1991 Experiments. Simple linear regression analyses were run for damage rating data as a function of nicotine levels (% of the wet weight of the leaf sample) for data from Tifton and Florence. The nicotine analyses provided significant correlations at both locations (Fig. 1), where nicotine levels explained 41% (r = 0.64, df = 16, $P < 0.01$) and 27% (r = 0.54, df = 16, $P < 0.05$) of the variation in damage ratings at Tifton and Florence, respectively. The slopes of these regression lines were not significantly different ($P < 0.005$) at the two locations.

Simple linear regression analyses also were run for damage rating data as a function of total cembrane diterpenes (% wet weight) for data from Tifton and Florence. Cembranes were analyzed because of their known stimulatory effects on oviposition by tobacco

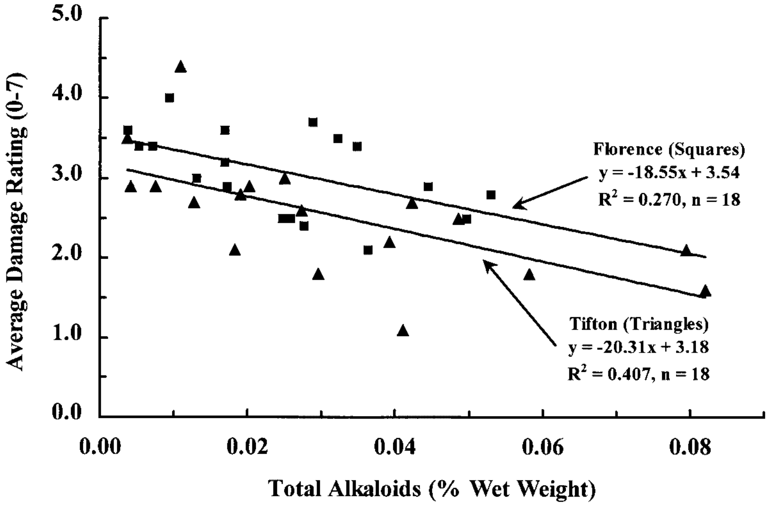

Fig. 1. Correlations between levels of total alkaloids (% wet weight) in the upper leaves of 18 tobacco entries and average damage ratings (rated 0–7, see text) for tobacco budworm larvae 7 wk after transplanting at Tifton, GA, and Florence, SC, 1991.

budworm moths (Jackson et al. 1984, 1986, 1989, Severson et al. 1991). Nicotine levels have a negative effect on survival of tobacco budworm larvae, whereas cembranes cause increased oviposition, thus having a positive influence on larval infestation levels and subsequent damage. However, levels of cembranes in these experiments were not significantly different among the 18 tobacco entries, indicating that these components did not differentially affect tobacco budworm oviposition at Tifton or Florence in 1991.

For the artificial infestations of the Oxford plots with second instar tobacco budworms at week 6, there was a significant negative correlation (r = 0.61, df = 16, $P < 0.01$) between average larval weight and alkaloid levels on the 18 tobacco isolines, but no significant correlation (r = 0.29, df = 16, $P < 0.05$) between average larval weight and alkaloids for the 8-wk measurement (Fig. 2). Larval survival was not significantly affected by alkaloid levels at either 6 or 8 wk. Cembranes had no effect on either survival or average weight of budworm larvae in the Oxford experiments (data not shown). Because larvae were placed on the plants, the cembranes did not function as ovipositional stimulants in this experiment.

Total alkaloids in the upper leaves sampled at Oxford remained at low levels until the plants were

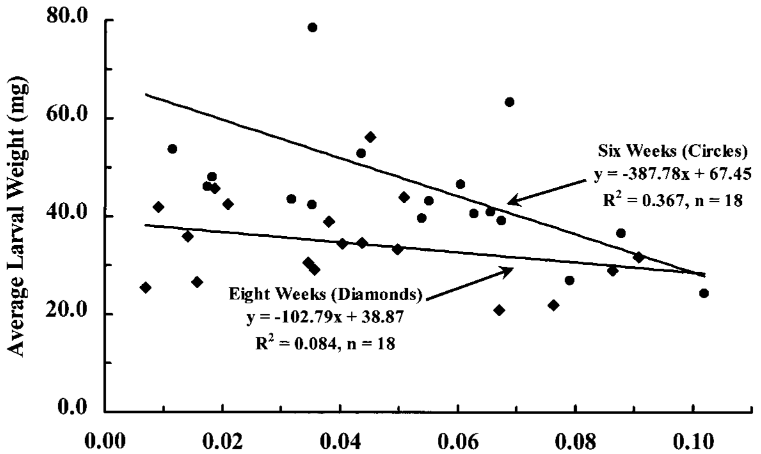

Fig. 2. Correlations between levels of total alkaloids (% wet weight) and average weight of tobacco budworm larvae allowed to feed for one wk on the upper leaves of 18 tobacco entries starting at 6 or 8 wk after transplanting at Oxford, NC, 1991. Each data point represents 21–55 larvae; initially, 75 larvae were placed on each tobacco entry for each experiment.

Table 1. Average survival of tobacco budworm larvae after being caged for 1 wk, starting as second instars, on different leaf positions of four isogenic tobacco lines beginning at 4, 8, 12, and 16 wk after transplanting at Oxford, NC, 1992

Week	Leaf position[a]	Cultural practice[b]	% Survival (± SE)				
			C-5	LN-4	LN-7	NC95	All tobacco entries
4	a	Before topping	81.3 ± 6.3ns[c]	78.7 ± 5.7a[d]	68.0 ± 7.5a[d]	57.3 ± 6.7ns[c]	71.3 ± 3.4a[e]
	b	Before topping	64.0 ± 7.4	61.3 ± 6.3b	45.3 ± 7.2b	40.0 ± 5.9	52.7 ± 3.5b
8	a	Before topping	70.7 ± 5.5a	77.3 ± 5.5a	61.3 ± 5.3a	61.3 ± 6.6a	67.7 ± 2.9a
	b	Before topping	49.3 ± 7.5b	41.3 ± 7.9b	5.3 ± 2.4b	1.3 ± 1.3b	24.3 ± 3.9b
	c	Before topping	29.3 ± 6.1c	14.7 ± 5.7c	1.3 ± 1.3b	1.3 ± 1.3b	11.7 ± 2.6c
	d	Before topping	12.0 ± 5.1d	2.7 ± 1.8c	2.7 ± 1.8b	0.0 ± 0.0b	4.4 ± 1.5d
12	a	Ratooned	70.0 ± 5.7a	59.0 ± 7.6a	29.0 ± 5.9a	36.0 ± 8.0a	48.5 ± 3.9a
	a	Topped	26.0 ± 6.0b	19.0 ± 5.7b	5.0 ± 3.5b	4.0 ± 2.3b	13.5 ± 2.5b
	b	Topped	22.0 ± 4.1b	18.0 ± 4.1b	2.0 ± 1.4b	6.0 ± 2.9b	12.0 ± 1.9b
	c	Topped	26.0 ± 6.2b	20.0 ± 5.8b	2.0 ± 1.4b	0.0 ± 0.0b	12.0 ± 2.5b
	d	Topped	28.0 ± 6.4b	13.0 ± 5.9b	1.0 ± 1.0b	0.0 ± 0.0b	10.5 ± 2.5b
	e	Topped	19.0 ± 4.7b	15.0 ± 5.4b	0.0 ± 0.0b	0.0 ± 0.0b	8.5 ± 2.0b
16	a	Ratooned	81.3 ± 5.3a	65.3 ± 5.0a	88.0 ± 4.3a	80.0 ± 6.5a	78.7 ± 2.8a
	c	Ratooned	41.3 ± 8.2b	28.0 ± 8.0b	24.0 ± 6.5b	8.0 ± 2.6b	25.3 ± 3.6b
	a	Topped	41.3 ± 7.7b	22.7 ± 7.8b	2.7 ± 2.7c	6.7 ± 3.2b	18.3 ± 3.5b
	c	Topped	54.7 ± 7.4b	25.3 ± 8.2b	6.7 ± 3.2c	6.7 ± 5.4b	23.3 ± 4.0b
All weeks and leaf positions			43.3 ± 2.0	33.9 ± 2.1	19.9 ± 1.9	18.0 ± 1.8	28.8 ± 1.0

[a] a, bud leaves; b, leaf 5 or 6 from the leaf bud or from the top of the plant; c, leaf 9 or 10 from the top of the plant; d, leaf 13 or 14 from the top of the plant; and e, leaf 17 or 18 from the top of the plant.
[b] 9 wk after transplanting, plants were either topped normally or cutoff-and-regrown (ratooned).
[c] Nonsignificant F value for leaf position effects according to analysis of variance.
[d] For each week, means in the same column followed by the same letter are not significantly different according to Fisher's least significant difference test, $P = 0.05$.

topped at week 10. Thereafter, there was a steady increase in the levels of total alkaloids in the upper leaves of the three isolines. However, the levels of alkaloids in the upper leaves of the cutoff-and-regrown (ratooned) plants remained very low. Overall, nicotine represented ≈97.9% of the pyridine alkaloids in the first year of this study. The other alkaloids measured were nornicotine, anabasine, and anatabine.

1992 Experiments. Overall ANOVAs (combined for all weeks) for larval survival and for larval weight gain showed significant effects for tobacco entry (larval survival, $F = 98.18$, df = 3, 994, $P < 0.0001$; weight gain, $F = 11.57$, 493, df = 3, $P < 0.0001$), leaf position (larval survival, $F = 129.35$, df = 7, 994, k$P < 0.0001$; weight gain, $F = 16.62$, df = 7, 493, $P < 0.0001$), and week (i.e., week that field bioassay was performed) (larval survival, $F = 211.96$, df = 3, 994, $P < 0.0001$; weight gain, $F = 83.57$, df = 3, 493, $P < 0.0001$). In addition, the interaction terms entry*replication (larval survival, $F = 5.55$, df = 9, 994, $P < 0.0001$; weight gain, $F = 2.84$, df = 9, 493, $P < 0.0029$) and week*replication (larval survival, $F = 5.92$, df = 6, 994, $P < 0.0001$; weight gain, $F = 5.58$, df = 6, 493, $P < 0.0001$) were significant for both parameters, whereas week*leaf position was significant for larval survival ($F = 16.15$, df = 5, 994, $P < 0.0001$), and entry*week was significant for larval weight ($F = 9.06$, df = 9, 493, $P < 0.0001$). All other interaction terms were not significant at the 5% level. Overall, tobacco budworm survival and larval weights were highest on the low alkaloid lines (C-5 and LN-4) and declined with the higher alkaloid lines (LN-7 and NC 95) (Tables 1 and 2). Larval survival was highest on the bud leaves and decreased as one moved down the tobacco stalk.

Subsequent ANOVAs for larval survival and larval weight were done for each week of testing (4, 8, 12, and 16). For the survival data, there were significant effects of tobacco entry (week 4, $F = 5.62$, df = 3, 102, $P < 0.0013$; week 8, $F = 27.08$, df = 3, 210, $P < 0.0001$; week 12, $F = 70.45$, df = 3, 429, $P < 0.0001$; week 16, $F = 21.30$, df = 3, 210, .$P < 0.0001$) and leaf position (week 4, $F = 15.31$, df = 1, 102, $P < 0.0002$; week 8, $F = 150.04$, df = 2, 210, $P < 0.0001$; week 12, $F = 70.70$, df = 5, 429, $P < 0.0001$; week 16, $F = 103.73$, df = 3, 210, $P < 0.0001$). Before the plants were topped (weeks 4 and 8) overall survival declined significantly as larvae fed further down the plant (Table 1). However, after topping (weeks 12 and 16), there was no significant difference in larval survival among leaf positions on the topped plants (week 12, $F = 0.82$, df = 4, 388, $P < 0.5101$; week 16, $F = 1.05$, df = 1, 112, $P < 0.3085$), however, survival was significantly higher on the bud leaves of the ratooned plants (Table 1). The pattern for larval weights was similar (data not shown). Levels of total alkaloids were significantly higher at lower stalk positions for weeks 4, 8, and 12 (week 4, $F = 8.04$, df = 1, 14, $P < 0.0132$; week 8, $F = 11.61$, df = 3, 39, $P < 0.0001$; week 12, $F = 12.47$ df = 4, 50, $P < 0.0001$) (Table 3), however at week 16 (8 wk after topping), there was no significant difference in alkaloid levels for leaf positions a and c of the topped plants (week 16, $F = 3.78$, df = 1, 17, $P < 0.0687$) (Table 3).

Additional ANOVAs and mean separations (LSD) were conducted for each entry for each week. At 4 wk after transplanting, there were significant differences in larval survival between leaf positions for LN-4 ($F = 4.28$, df = 1, 24, $P < 0.0495$) and LN-7 ($F = 4.31$, df = 1, 24, $P < 0.0487$), but not for the other two tobacco

Table 2. Average weight of tobacco budworm larvae after being caged for 1 wk, starting as second instars, on different leaf positions of four isogenic tobacco lines beginning at 4, 8, 12, and 16 wk after transplanting at Oxford, NC, 1992

Week	Leaf position[a]	Cultural practice[b]	Weight, mg (± SE)				
			C-5	LN-4	LN-7	NC95	All tobacco entries
4	a	Before topping	187.7 ± 22.4a[c]	190.2 ± 16.1ns[d]	59.3 ± 7.8 ns[d]	76.9 ± 11.1ns[d]	128.5 ± 10.9a[c]
	b	Before topping	119.6 ± 16.3b	155.7 ± 13.4	61.7 ± 10.5	82.9 ± 8.7	106.0 ± 7.6b
8	a	Before topping	77.1 ± 11.6ab	73.9 ± 5.9ab	104.3 ± 14.6a	49.1 ± 5.2a	76.1 ± 5.6a
	b	Before topping	80.4 ± 14.0a	121.2 ± 19.2a	9.5 ± 29.9b	8.0[e]b	86.0 ± 12.0ab
	c	Before topping	51.8 ± 6.5ab	55.5 ± 16.6ab	7.0[e] b	9.0[e]b	50.5 ± 6.6bc
	d	Before topping	39.6 ± 11.8b	18.0 ± 5.0b	9.0 ± 10.0b	—[f]	28.0 ± 7.8c
12	a	Ratooned	64.1 ± 13.2ns	90.3 ± 12.2a	72.2 ± 12.9a	67.9 ± 13.7a	73.8 ± 6.6a
	a	Topped	54.6 ± 10.3	32.0 ± 10.9c	20.5 ± 7.5b	14.7 ± 3.3b	40.3 ± 6.7b
	b	Topped	41.5 ± 10.4	69.1 ± 15.6ab	14.5 ± 7.5b	13.3 ± 36.7b	55.3 ± 8.8ab
	c	Topped	63.9 ± 13.8	50.4 ± 12.2bc	34.0 ± 20.0b	—[f]	55.8 ± 8.7ab
	d	Topped	72.3 ± 18.5	42.5 ± 7.1bc	19.0[e]b	—[f]	60.0 ± 12.3ab
	e	Topped	50.7 ± 9.5	42.6 ± 7.6bc	—[f]	—[f]	47.7 ± 6.5b
16	a	Ratooned	66.5 ± 6.7a	99.7 ± 19.0a	71.1 ± 10.8a	58.9 ± 8.6a	74.0 ± 6.3a
	c	Ratooned	41.8 ± 5.7b	60.8 ± 21.0ab	37.3 ± 17.7b	20.0 ± 7.0b	42.4 ± 7.5b
	a	Topped	31.5 ± 6.9b	21.7 ± 4.1b	16.0[e]b	15.5 ± 3.0b	25.6 ± 3.9b
	c	Topped	35.2 ± 6.1b	21.9 ± 3.6b	9.8 ± 18.9b	22.0 ± 9.0b	27.0 ± 3.7b
All weeks and leaf positions			69.2 ± 4.1	86.2 ± 5.3	59.7 ± 4.8	59.1 ± 4.3	70.9 ± 2.7

[a] a, bud leaves; b, leaf 5 or 6 from the leaf bud or from the top of the plant; c, leaf 9 or 10 from the top of the plant; d, leaf 13 or 14 from the top of the plant; and e, leaf 17 or 18 from the top of the plant.
[b] 9 wk after transplanting, plants were either topped normally or cutoff-and-regrown (ratooned).
[c] For each week, means in the same column followed by the same letter are not significantly different according to Fisher's least significant difference test, $P = 0.05$.
[d] Nonsignificant F value for leaf position effects according to analysis of variance.
[e] One surviving larva (cannot compute SE).
[f] No surviving larvae.

entries (Table 1). There was a significant difference in larval weights only for C-5 at week 4 ($F = 19.18$, df = 1, 23, $P < 0.0002$) (Table 2). At 8 wk after transplanting, larval survival and larval weight declined significantly with lower leaf positions. For the two tobacco entries with the lowest levels of alkaloids (C-5 and LN-4; Table 3) at week 8, survival declined significantly at each lower leaf position (C-5, $F = 20.92$, df = 3, 48, $P < 0.0001$; LN-4, $F = 34.24$, df = 3, 48, $P < 0.0001$) (Table 1). However, for the two tobacco entries with the highest levels of alkaloids (LN-7 and NC 95; Table 3) at week 8, survival was only significantly higher at the top leaf position, and there were no significant differences in survival rates among the lower leaf po-

Table 3. Levels (% wet weight) of total alkaloids at different leaf positions on four isogenic tobacco lines at 4, 8, 12, and 16 wk after transplanting at Oxford, NC, 1992

Week	Leaf posiion[a]	Cultural practice[b]	% Wet weight (± SE)				
			C-5	LN-4	LN-7	NC95	All tobacco entries
4	a	Before topping	0.018 ± 0.002ns[c]	0.027 ± 0.001ns[c]	0.097 ± 0.006b[d]	0.137 ± 0.015ns[c]	0.025 ± 0.005ns[c]
	b	Before topping	0.016 ± 0.002	0.030 ± 0.002	0.177 ± 0.012a	0.163 ± 0.008	0.061 ± 0.008
8	a	Before topping	0.007 ± 0.002b	0.009 ± 0.002c	0.044 ± 0.002c	0.043 ± 0.005d	0.028 ± 0.005d
	b	Before topping	0.007 ± 0.001b	0.016 ± 0.005c	0.140 ± 0.031bc	0.127 ± 0.021c	0.078 ± 0.016c
	c	Before topping	0.009 ± 0.000b	0.033 ± 0.001b	0.295 ± 0.073ab	0.276 ± 0.017b	0.159 ± 0.035b
	d	Before topping	0.014 ± 0.001a	0.046 ± 0.003a	0.385 ± 0.074a	0.465 ± 0.018a	0.255 ± 0.052a
12	a	Ratooned	0.012 ± 0.001bc	0.025 ± 0.003bc	0.114 ± 0.018d	0.105 ± 0.007d	0.049 ± 0.012d
	a	Topped	0.008 ± 0.002cd	0.021 ± 0.007c	0.341 ± 0.039c	0.199 ± 0.048c	0.145 ± 0.035c
	b	Topped	0.006 ± 0.001d	0.020 ± 0.007c	0.381 ± 0.050c	0.157 ± 0.020cd	0.157 ± 0.038c
	c	Topped	0.013 ± 0.004b	0.045 ± 0.018bc	0.576 ± 0.022b	0.324 ± 0.009b	0.263 ± 0.058b
	d	Topped	0.014 ± 0.001b	0.056 ± 0.003ab	0.665 ± 0.084b	0.412 ± 0.014a	0.307 ± 0.067b
	e	Topped	0.028 ± 0.004a	0.087 ± 0.012a	0.851 ± 0.033a	0.460 ± 0.009a	0.381 ± 0.082a
16	a	Ratooned	0.003 ± 0.001c	0.007 ± 0.001b	0.045 ± 0.006c	0.047 ± 0.012c	0.025 ± 0.007c
	c	Ratooned	0.011 ± 0.002b	0.028 ± 0.001b	0.190 ± 0.038c	0.166 ± 0.012b	0.099 ± 0.026b
	a	Topped	0.020 ± 0.001a	0.182 ± 0.043a	0.884 ± 0.089b	0.479 ± 0.029a	0.391 ± 0.102a
	c	Topped	0.015 ± 0.002a	0.137 ± 0.031a	1.213 ± 0.025a	0.559 ± 0.058a	0.481 ± 0.142a
All weeks and leaf positions			0.012 ± 0.001	0.048 ± 0.007	0.400 ± 0.050	0.257 ± 0.025	0.182 ± 0.018

[a] a, bud leaves; b, leaf 5 or 6 from the leaf bud or from the top of the plant; c, leaf 9 or 10 from the top of the plant; d, leaf 13 or 14 from the top of the plant; and e, leaf 17 or 18 from the top of the plant.
[b] 9 wk after transplanting, plants were either topped normally or cutoff-and-regrown (ratooned).
[c] Nonsignificant F value for leaf position effects according to analysis of variance.
[d] For each week, means in the same column followed by the same letter are not significantly different according to Fisher's least significant difference test, $P = 0.05$.

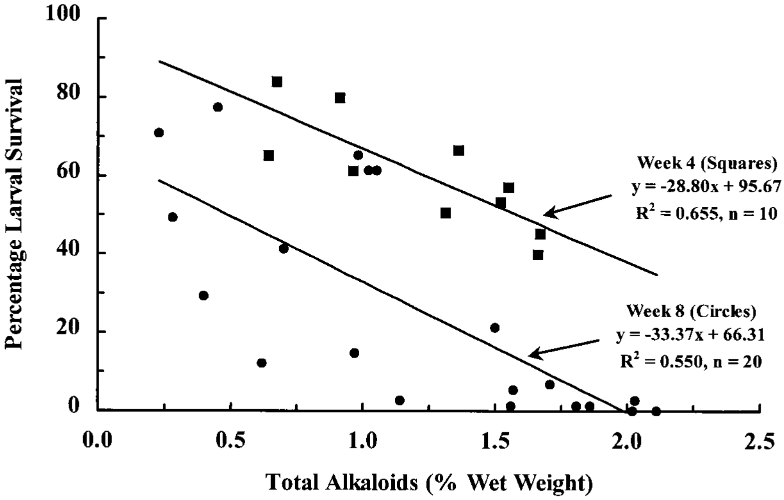

Fig. 3. Correlations between percentage survival of tobacco budworm larvae and levels of total alkaloids (% wet weight) in four isogenic tobacco lines at various leaf positions at four and 8 wk after transplanting at Oxford, NC, 1992. Data points represent the average of 15 larvae.

sitions (Table 1). In general, larval weights followed a similar pattern at week 8 (Table 2).

For all tobacco entries at 12 and 16 wk after transplanting (4 and 8 wk after topping or ratooning), survival rates of tobacco budworm larvae were similar at all leaf positions on the topped plants (Table 1). However, survival rates were significantly higher (week 12, $F = 213.05$, df = 1, 420, $P < 0.0001$; week 16, $F = 50.35$, df = 1, 204, $P < 0.0001$; orthogonal comparisons of all topped versus all ratooned plants) on the bud leaves of the ratooned plants than on the lower leaves of the topped plants (Table 1). A similar pattern existed for larval weights for the high-alkaloid tobacco entries (Table 2).

At 4 wk after transplanting, alkaloid levels for the two leaf positions only differed for LN-7 (Table 3). However, by week 8, alkaloid levels generally increased at lower leaf positions for all of the entries except C-5, where alkaloid levels were quite low at all leaf positions. This pattern continued for week 12, but by week 16 alkaloid levels in the uppermost leaf position of the topped plants were similar to those in the lower leaf position (Table 3). Overall, nicotine represented 98.5% of the pyridine alkaloids quantified in 1992.

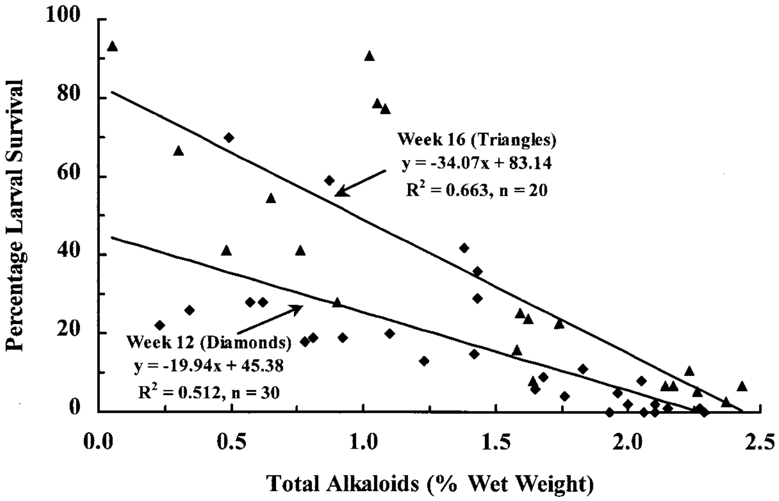

Fig. 4. Correlations between percentage survival of tobacco budworm larvae and levels of total alkaloids (% wet weight) in four isogenic tobacco lines at various leaf positions at 12 and 16 wk after transplanting at Oxford, NC, 1992. Data points represent the average of 15 larvae.

Regression analyses were run for data combined for all tobacco entries and leaf locations for each week. Significant correlation coefficients were determined for each week (Figs. 3 and 4). An analysis for the homogeneity of regression coefficients showed that the slopes of the regression lines were not significantly different ($P < 0.005$) for week 4 (-28.8), week 8 (-33.4), and week 16 (-34.1) (Figs. 3 and 4).

Discussion

We have demonstrated a relationship between the distribution of alkaloids in tobacco and survival and development of tobacco budworm larvae. Correlations of alkaloid levels in tobacco field plots with larval survival and development indicate that even small amounts of these toxins can have a measurable negative effect. Controlled field bioassays further demonstrated that larval survival and weight gain were correlated with alkaloid levels in these isogenic lines. We also showed that during the growing season, especially before the plants are topped, alkaloid levels increase as one moves down the stalk, and correspondingly, larval survival and weight gain is diminished at the lower leaf positions.

Although tobacco budworm moths oviposit most of their eggs on flue-cured tobacco leaves near the leaf bud (Neunzig 1969, Jackson et al. 1983), larvae feed little on fully expanded tobacco leaves. Instead, most neonates chew through the egg chorion and proceed to the leaf bud (Mistric and Smith 1969). Neonates feed intermittently on leaves on their way to the leaf bud, but they typically stay in the bud especially in hot, dry weather (Mistric and Smith 1974). Alkaloid levels in the upper leaves most favored by ovipositing tobacco budworm moths are comparatively low and have a limited effect on larval survival and development, even in the high alkaloid lines we tested. Few tobacco budworm eggs are deposited on leaves further down the stalk or after topping, and few early instars are found on these ripening leaves in the field (Mistric and Smith 1969, 1974; Neunzig 1969). Young larvae that move down the plant have little chance of survival. Therefore, it appears that tobacco budworm larvae escape nicotine poisoning by not feeding on plant parts with high alkaloid levels.

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